AD________________

GRANT NUMBER DAMD17-94-J-4368

TITLE: pH Regulation by Breast Cancer Cells in Vitro and in Vivo

PRINCIPAL INVESTIGATOR: Robert J. Gillies, Ph.D.

CONTRACTING ORGANIZATION: University of Arizona
Tucson, Arizona 86719

REPORT DATE: September 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1998	Annual (15 Aug 97 - 14 Aug 98)

4. TITLE AND SUBTITLE
pH Regulation by Breast Cancer Cells in Vitro and in Vivo

5. FUNDING NUMBERS
DAMD17-94-J-4368

6. AUTHOR(S)
Robert J. Gillies, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Arizona
Tucson, Arizona 86719

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

19990804 096

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The above grant has completed its final year of funding and is currently on a 1-year no-cost extension. In the past year, many avenues of investigation have come to completion, and we are in the process of pushing manuscripts through the publication process. This annual report will therefore be a departure from previous ones in that it will be organized as an annotated list of manuscripts citing support from the current grant. Thirteen manuscripts are described. A full set of completed papers will be submitted as the final report. Continuance of this work will be pursued with funding from the NIH, and this is described in the last section.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

✓ Where copyrighted material is quoted, permission has been obtained to use such material.

✓ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

✓ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

✓ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Robert Gillies 9/12/98

PI - Signature Date

TABLE OF CONTENTS

ANNUAL REPORT
USAMRMC DAMD17-94-J-4368

Abstract: The above grant. "pH regulation by breast cancer cells in vitro and in vivo" has completed its final year of funding and is currently on a 1-year no-cost extension. This rate of expenditure was pre-planned. In the last year, many avenues of investigation have come to completion, and we are in the process of pushing manuscripts through the publication process. This annual report will therefore be a departure from the previous in that it will be organized as an annotated list of manuscripts citing support from the current grant. A full set of completed papers will be submitted as the final report. Continuance of this work will be pursued with funding from the NIH, and this is described in the last section.

Work directly supported by the current grant:

1. **Martinez-Zaguilan R, Martinez GM, Gomez A and Gillies RJ (1998) Distinct regulation of pH and Ca by cells of differing metastatic potential.** ***J. Cell. Physiol*** **176:196-205.** This paper describes the occurrence of Vacuolar-type H^+ ATPases as a significant contributor to pH regulation in metastatic, compared to non-metastatic breast cancer and melanoma cells. We hypothesize at this juncture that the enhanced V-ATPase activity might result from rapid turnover of acidic vesicles, and not V-ATPase statically resident in the plasma membrane (see manuscripts 4 and 5 below).

2. **Raghunand N, Altbach MI, van Sluis R, Baggett B, Taylor CW, Bhujwalla ZM and Gillies RJ (1998) Plasmalemmal pH-gradients in Drug-Sensitive and Drug-Resistant MCF-7 Human Breast Carcinoma Xenografts Measured by ^{31}P MR Spectroscopy.** ***Biochemical Pharmacology*** **(in press).** This paper uses NMR to monitor the intra and extracellular pH of tumor xenografts of human breast cancer cells in SCID mice. It describes the observation that the extracellular pH in tumors from the drug-resistant lines is significantly lower than that from the drug-sensitive lines.

3. **van Sluis R, Bhujwalla Z, Raghunand N, Ballesteros P, Alvarez J. Cerdan S Gillies RJ (1998) Imaging of extracellular pH of tumors using 1H MRSI.** ***Magnetic Resonance in Medicine*** **(in press).** This paper describes a novel pH measurement technique that allows us to image the extracellular pH of tumors non-invasively in vivo. The technique is based on magnetic Resonance Spectral Imaging and employs a novel imidazole derivative developed by Drs. Cerdan and Ballesteros.

4. **Raghunand N, Martinez-Zaguilan R and Smith DJ, Martinez GM, Rojas B, Dalton WS, Mahoney B and Gillies RJ, (1998) pH and drug resistance I. Drug-resistant breast cancer cells express plasma membrane V-ATPase.** ***Biochemical Pharmacology*** **(in press).** This paper is similar to MS #1, above, in that the mechanisms of pH regulation are compared between cells of different phenotypes. In this case, we compared drug-resistant and drug-sensitive MCF7 human breast cancer cells and observed that there were no significant differences between bicarbonate transport, Na/H exchange or resting pH in the cytosol or in the endosomes between these cell lines. however, the drug-resistant lines (both P-gp positive and –negative) had significant V-ATPase activity. We further postulated that this resulted from turnover of acidic vesicles and to support that hypothesis, showed that endosomal turnover rates were much more rapid in the drug-resistant lines,

compared to the drug-sensitive lines. Furthermore, the drug-resistant lines trapped weakly basic chemotherapeutic agents (i.e. doxorubicin) into acidic vesicles, whereas the drug-sensitive cells did not.

5. **Raghunand N, Wright SH and Gillies RJ (1998) pH and drug resistance II. Turnover of acidic vesicles and resistance to weakly basic chemotherapeutics. *Biochemical Pharmacology* (in press).** This paper is a companion to the previous and explores, mathematically, the potential involvement of acidic vesicles in resistance to weakly basic chemotherapeutic drugs. It diverged from previous models in that it incorporated the turnover of vesicles and the facilitated transport of charged drug (e.g. organic cation transport, OCT). This model shows, for the first time, that acidic vesicles are equally effective as MDR-1 in conferring drug resistance, if the vesicles turn over and if there is significant OCT. We are currently following this up using RT-PCR of drug resistant cells for the presence of an OCT isoform.

6. **Raghunand N, He X, van Sluis R, Mahoney B, Baggett B, Taylor C, Roe D, Bhujwalla Z and Gillies RJ (1998) Enhancement of Chemotherapy by Manipulation of tumor pH. *Br. J. Cancer* (MS submitted, Ref. No. TH/1998/004192).** In this paper, we examine the hypothesis that the acidic extracellular environment in tumors can contribute to resistance to weakly basic chemotherapeutic drugs. This hypothesis is supported by a preponderance of in vitro data. Using in vivo NMR, we show, for the first time, that supplementation of drinking water with bicarbonate causes significant and substantial increases in the extracellular pH of tumors in vivo and that tumors thus treated are more sensitive to killing by doxorubicin. We plan to follow this up using a syngeneic system, which can tolerate higher doses of doxorubicin without compromising the animals' health.

7. **Raghunand N, van Sluis R, Altbach MI, Aiken NR, Bhujwalla ZM, Stubbs M and Gillies RJ (1997) Measurment of extracellular pH excursions in breast cancer xenografts: deconvolution of T2* effects. *Magnetic Resonance in Medicine.* (MS in preparation).** In earlier work, we developed a method to monitor the extracellular pH of tumors using an exogenous compound, 3-aminopropylphosphonate (3-APP). This work investigates the shape and width of the 3-APP resonance to render an estimate of the pH heterogeneity in tumors. This is a significant problem because many researcher feel that acid pH promotes carcinogenesis. This method will yield an estimate of the fraction of the tumor cells which are in the lowest pH environments.

8. **van Sluis R, Bhujwalla Z, Raghunand N, Ballesteros P, Alvarez J. Cerdan S Gillies RJ (1998) Manipulation of tumor pH observed with novel pH imaging methodology. *Nature* (MS in preparation).** This paper applies the technique described in MS #3 to time-resolved images of tumor pH during imposed acidic (via carbogen) and basic (via bicarbonate) loads

9. **Galons JP, Altbach MI, Paine-Murrieta G, Taylor C and Gillies RJ. Chemotherapeutic Response Monitored by Diffusion Weighted MRI. *Magnetic Resonance in Medicine* (MS in preparation).** This manuscript describes the effect of Taxol chemotherapy on drug-sensitive and drug-resistant breast cancer tumors in vivo. We have used diffusion-weighted MRI to monitor the effect of taxol on individual tumors. This is an important study because the DWI effects can be observed within hours of taxol administration, whereas frank changes

in tumor volume may not be apparent for days. Furthermore, conventional caliper measurements of tumor volume are compromised by a complex tumor morphology, much of which may be acellular (i.e. edema or lipid deposits). Thus, MRI provides not only an early indicator of response, but also much more accurate morphometry. This approach will be followed up clinically/

Work done in collaboration with other workers:

10. **Martinez-Zaguilan R, Seftor EA, Seftor RE, Chu YW, Gillies RJ, and Hendrix MJ (1996) Acidic pH enhances the invasive behavior of human melanoma cells.** ***Clinical & Experimental Metastasis*****, *14*:176-86.** This manuscript describes the effect of acidic conditions on the migration and invasion of highly- and lowly-metastatic melanoma and breast cancer cells in vitro. It reports that growth at acidic conditions consistently renders all cells more invasive. In the case of melanoma cells, the lowly metastatic A375p cells grown at acidic pH (6.7) exhibited migration and invasion comparable to the highly metastatic C8161 cells grown at alkaline pH (7.4). These data support the hypothesis that metastasis might be augmented by hostile tumor environments (e.g. low pH).

11. **Xie X, Gillies RJ and Gerner EW (1997) Characterization of a diamine exporter in Chinese hamster ovary cells and identification of specific polyamine substrates.** ***J. Biol. Chem.*** ***272*****:20484-20489.** This paper describes the initial characterization of a novel polyamine exporter, which exchanges polyamine (organic cations) for protons. Such an activity may play a role in drug resistance, by transporting organic cations (e.g. anthracyclines) out of the cytosol and into recycling vesicles (see MS # 5).

12. **Bhujwalla ZM, McCoy CL, Glickson JD, Gillies RJ and Stubbs M (1998) Non-invasive estimations of intra- and extracellular volume and pH by 31-P MRS: applications to untreated and 5-FU treated RIF-1 tumors.** ***Br. J. Cancer.*** **(in press).** This paper describes the in vivo MR measurements of tumor cell volume using a combination of 3-APP, which is impermeant) and dimethylmethylphosphonate (DMMP), which is freely permeant. Such an approach was used previously by us in vitro, but this is the in vivo first report. Tumors that respond to 5-FU have significant and consistent reductions in their intracellular volumes.

13. **Bhujwalla ZM, Aboagye EO, Gillies RJ, Mendola CE and Backer JM (1998) Nm23-transfected MDA-MB-435 human breast carcinoma cells form tumors with altered phospholipid metabolism and pH. A ^{31}P NMR study In vivo and In vitro.** ***Br. J. Cancer.*** **(submitted).** NM-23 is a controversial family of proteins whose expression is correlated with reduced metastatic potential. In this study, we transfected highly metastatic MDA-mb-435 cells to express these proteins (2 isoforms, along with inactive mutant controls). When grown as tumor xenografts in SCID mice, the non-metastatic transfectants had significant alterations in their 31-P MR profiles, viz. significant alterations in both extracellular pH and phosphodiester levels. This is the first indication that the extracellular pH of tumors from metastatic cells may have a fundamental different pH environment than non-metastatic cells.

A grant to the NIH for further support of this research has been submitted. CA77575-01a1, "Causes and consequences of Acid pH in Tumors" has been submitted and reviewed by the diagnostic radiology study section and received a score in the 32nd percentile. The major criticism of this proposal was that the technique outlined in manuscript #3 was not yet installed on our new NMR machine (which arrived in March, 1998). The proposal will be resubmitted Nov. 1, 1998.